IN VITRO HEPATOPROTECTIVE ACTIVITY FROM LEAF PART OF "*PICRORHIZA KURROA*"AND "*INULA RACEMOSA*" AND ITS HERBAL FORMULATION

SYNOPSIS

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1. INTRODUCTION

Herbal plants have been used in the health maintenance customs since the origin of mankind. According to an estimate made by the World Health Organization (W.H.O.) traditional medicine forms the basis of primary healthcare of about 80% population of the world. Primary reason for that being the inexpensive nature of herbal medicines as compared to modern pharmaceutics as these can be grown from seed or gathered from nature for little or no cost. *Picrorhiza kurroa* Royle ex Benth is one of the most established herbal plants with extraordinary medicinal properties. This drug is known to be in medicinal use since 5000 years ago (Bhramanand, 2015). It belongs to Scrophulariaceae family and commonly called as Kutki & is a well-known herb for its hepatoprotective action and its wide range of pharmacological activities. Katuki is known to help in removing the excess

Fig. 1 :Leaves and Flowering Part of *Picrorhiza kurroa*

fire energy from our body thus acting as a cooling agent. It balances pitta and kapha which can cause acidity, digestive problems and fat. So, it helps to improve digestion, metabolism of carbohydrates, proteins and fats. With improved metabolism various problems like increased level of urea, creatinine, diabetes, heat and hyperthyroidism can be managed (Lucas, 2013). Katuki has been one of the important sources of Ayurvedic medicine as well as modern medicine. Although it's majorly used for liver disorders, but its active components present in

various parts of plant are providing relief and saving millions of life from the very ancient time. Due to wide spectrum of biological activities this plant is widely used in drug industries (Raina D & Raina S 2021).

1.1.Vernacular Names

The word Picrorhiza is made up of two words "picross", Greek word which indicates a meaning bitter while the word "rhiza" indicates roots. The Punjabi name of the plant "Karu", indicates the same meaning as that of "rhiza" (Masood *et al.*, 2015). Scrophulariaceae family consists of 200 genera and 3000 species which are mainly found in the northern temperate regions of the world. Popular garden plants (including tiny alpines) and aesthetically important plants such as Penstemon, Mimulus and Calceolaria are some members of this family (Bhattacharjee *et al.,* 2013). The regional names of Katukiin India are shown below:

Table 1: Vernacular Names of *Picrorhiza kurroa*

English	Hellebore
Sanskrit	Kutki
Hindi	Tikta, Tiktarohini, Kaurohini, Kavi, Sutiktaka, Kauka, Rohini
Assamese	Katki, Kutki
Gujrati	Kadu, Katu
Kannada	Katuka rohini,
Malyalam	Katuka rohini, Kaduk rohini
Marathi	Kutki, Kalikutki
Oriya	Katuki
Punjabi	Karru, Kaur
Tamil	Katuka rohini, Katuku rohini, Kadugurohini
Telugu	Karukarohini
Urdu	Kutki

1.2. Botanical Description

Picrorhiza kurroa is a small perennial herb and fast depleting high-valued medicinal plant which is commonly known as Kutki , belongs to the family Scrophulariaceae **(**Prakash *et al.,* 2020).

Table 2: Taxonomy of *Picrorhiza kurroa*

Taxonomic Rank	Taxon
Kingdom	Plantae
Division	Dicotiledonae
Class	Asteride
Order	Scrophulariales
Family	Scrophulariaceae
Genus	*Picrorhiza*
Species	*Kurroa*
Common Name	Kutki

1.3. Habitat & Geographical Distribution

Picrorhiza kurroa an indigenous plant of western Himalayan at 3000-5000 m of elevation(Bishen & Mahendra, 1994). Western Himalayan range includes Jammu and Kashmir, Himachal Pradesh and Uttarakhand. In Kashmir, it is mainly found in Gurez, Lolab, Karna, Sindh and Lidder Valleys. In Uttarakhand it is found in areas of Badrinath, Kedarnath and Chamba. In Himachal Pradesh it is wildly found in hilly districts such as Chamba, Pangi, Kullu, Shimla, Kinnaur and Lahaul valleys (Thakur, 2015). *P. kurroa* grows well in moist, sandy clay loamin textured soils which have high inorganic matter present in them (Sharma *et al.,* 2015). It is found in Pakistan, Nepal, Bhutan, China (International Trade, 2007). It is commonly seen associated with the herbs like *Aconitumviol aceum*, *Lagotiscashmiriana*, *Potentillakashmirica*, *Sedum ewersii* and *Senecio jacquemontianus*.

1.4. Description of *Picrorhiza kurroa*

P. kurroa is a hairy herb with perennial herb which attains height upto 10-20 cm. A small leafy weak creeping stem is present which is erect at flowering. This plant contains 6-10 inches long hard roots which are wrinkled and grayish to brown in colour (Rasool *et al.,* 2016; Kar, 2007; Liu *et al.,* 2010).

a) Rhizomes:

Rhizomes are 2.5–12.0 cm long, 0.3-1.0 cm thick, cylindrical which are curved in irregular manner with the branching and rooting at the jointed nodes. The rhizomes are externally grayish-brown in appearance. Numerous aerenchymas are present in leaf, aerial stem and

rhizome. It has pleasant odor and bitter taste. Rhizomes are jointed and zigzag, cylindrical, irregularly curved with branching and rooting at the jointed nodes (Raghunathan & Mitra 1982). The rhizome consists of 20-25 layers of cork consisting of tangentially elongated, suberised cells; cork cambium is generally 1-2 layered; cortex single layered or absent, primary cortex persists in some cases, one or two small vascular bundles present in cortex; vascular bundles surrounded by single layered endodermis of thick-walled cells; secondary phloem composed of phloem parenchyma and a few scattered fibers; cambium 2-4 layered; secondary xylem consists vessels, tracheids, xylem fibers and xylem parenchyma, vessels vary in shape and size having transverse oblique articulation; tracheids long, thick-walled, lignified, more or less cylindrical with blunt tapering ends; xylem parenchyma thin-walled and polygonal in shape; centre occupied by a small pith consisting of thin-walled cells; simple round to oval, starch grains, measuring 25-104 μm in dia., abundantly found in all cells (Kirtikar & Basu 1987).

b) Root:

Roots are thin, cylindrical, 5-10cm long 0.05-0.1mm in diameter, mostly attached with rhizome; inner surface black with whitish xylem. Odor is pleasant and taste is bitter .They are greyish to brown in appearance, while the fracture is tough. Root stacks are irregularly curved as thick as the little finger (Elizabeth, 2002; Kirtikar & Basu 1999). Young root generally have single layered epidermis, some epidermal cells elongate forming unicellular hairs; hypodermis single layered; cortex is 8-14 layered consisting of oval to polygonal, thick-walled, parenchymatous cells; primary stele tetrarch to heptarch, enclosed by single layered pericycle and single layered, thick-walled cells of endodermis; mature root shows 4-15 layers of cork, 1-2 layers of cork cambium; secondary phloem poorly developed; secondary xylem consisting of vessels, tracheids, parenchyma and fibers; vessels have varying shape and size, some cylindrical with tail like, tapering ends, some drum shaped with perforation on end walls or lateral walls; tracheids cylindrical with tapering pointed ends; fibers aseptate, thick-walled, lignified with tapering blunt chisel-like pointed ends. Transverse section of root shows that periderm consist of about 8-10 layers of thin walled cork cells having average dimensions of 35μ × 20μ. Phellogen cells appear rectangular and radially flattened in transverse section and lack in intercellular spaces. The cells of phelloderm are more or less arranged definitely in radial rows. The cortex consists of thin parenchymatous cells with average dimension of 50μ

× 15µ.The secondary phloem consists of layers of cells and they originate from the cambium. The secondary phloem is composed of sieve tubes, companion cells and parenchyma. The phloem ray cells are indistinguishable. Xylem consists of groups of thick walled fibers and few porous tracheae, embedded in between the pith and the cortex. Trachae with porous wall thickening have an average diameter of 24µ. Cambium cells have average dimensions of 11µ × 4µ. Thepith is characterized by the presence of parenchymatous cells with porous wall thickening having diameter of 50µ × 40µ (IP 1966).

d) Cortex:

Cortex consists of collenchymatous and parenchymatous cells, cortical bundles, radially arranged xylem which is endarch, and the pith. A large number of root and leaf traces, leaf gaps and branch connection are visible. The vascular ringis not continuous and consists of 3-6 vascular area of differentsizes (Mitra & Prasad, 1972). The cells of cortex and pith are spongy and filled with starch grains (Mehra & Jolly, 1968). The roots show only thick parenchymatous cells and four to seven arcs of exarch xylem. Cortical bundles and pith are absent.

d) Leaves:

Leaves are 5-15 cm in length. Leaves are oval in shape and are acuminate, serrate, stalked, winged, oblanceolate or narrowly spathulate and are coarsely toothed. Two kind of glandular hair are present over the leaves.

e) Flowers:

Picrorhiza kurroa has white or pale purple colored bisexual flowers which are present on long terminal spikes. Calyx has equal 5 parts with 4-5 lobbed corolla. Lobes are actinomorphic. Slightly didynamous stamens which are 4 in number are inserted on corolla stamened form 6 mm. long with longer filaments 8mm. long, of the longer stamened from 6 mm with filaments 2 cm. Capitate stigma is present.

f) Fruits/Seeds:

Picrorhiza kurroa has acute ovoid and swollen fruits/capsules which are 6-10 mm in size, tapered at top, split up into 4 valves, stamened form 6 mm long with longer filaments 8 mm long, of the longer stamened from 6 mm with filaments 2 cm long ellipsoid in shape. A very thick and transparent seed coat is present over the seeds. Numerous pale brown seeds are present in this plant. Seeds are almost 1 x 0.8 mm in size and ellipsoid in shape.

Fig.2: Leaves of *Picrorhiza kurroa*

1.5. Agrotechnology

Propagation

Propagation of *P. kurroa* can be achieved sexually or asexually from seeds, rhizomes and stolons. To maintain quality and uniformity of the plant, it is propagated through stolon cuttings. Approximately 1.0 to 1.5 kg of seeds are required for raising saplings in1 hectare of land (Das, 2008).

Soil requirements

Sandy loam soil rich in organic carbon and porous humus texture is the best for its cultivation. Moisture level should be maintained but should not be over moist. Furthermore, cultivation under the small shrubs or partially shaded areas gives optimum yield (Caldwell *et al*., 2016).

Time of seed sowing and stolon cuttings

Seed sowing should be carried out during March-April in beds at a lower altitude, November-December in a green house and May in the alpine area. Seeds exhibit maximum

germination 52-58% when sown on the top layer of thesoil. Vegetative propagation through the stolon is more successful than seed sowing. Propagation through the stolon cutting reduces the crop duration. Most of the farmers propagate the *P. kurroa* through the stolon cuttings.

The rooted seedling can be transplanted in the month of February-March but if the crop is vegetatively propagated then transplanting can be done in the month of September-October. In areas where snow fall occurs, planting can be delayed till the snow melts (Chand, Malik,& Nautiyal, 2016).

Planting density and nutritional requirement

For better development of plant and harvesting of stolon, it is planted at 30×30 cm apart from row to row and plant to plant. Side stolon is harvested as a drug for consumption and marketing as the plant is perennial. Generally, 15 tons/ha manure is recommended at lower altitudes and 10 tons/ha at mid- altitudes to attain the best productivity for three years. Application of manure during the winter months and transplantation is carried out in the rainy season by digging a 6- inch deep channel . About 20 tons/ha of well decomposed farmyard manure (FYM) before transplanting should be applied. Since it is a perennial crop, supplemental doses of FYM may also be added during winter months (Kumar *et al.*, 2013).

Irrigation and weed control

For better survivability, regular irrigation is required to maintain the moisture. As per demand on the specific siteand under specific climate conditions watering once daily is necessary. At the lower altitude the plant requires frequent irrigation to decrease the mortality and for better survival. Plants need water at two days interval during the months of winter. To attain optimum production of the plant, weeds should be removed on regular basis. During early stages weeding should occur weekly and during later stages weeding can take place monthly (Caldwell *et al*., 2016; Chand, Malik, & Nautiyal, 2016).

Maturity and harvesting

At different altitudes, plants mature at different stages. They attain maturity when the completion of reproductive phase occurs during this time percentage of active compound is high. At lower altitude it occurs in September and within alpine zone it occurs in October. Stolons are harvested in the 3rd year of the planting. Harvested stolons with roots are removed from the mother plant but for regular growing the mother plant is kept undamaged. During the following rainy season, the harvested stolon which contains apical part with roots is removed from the remaining stolon and planted in the soil which will give rise to new plants. The stolon can be used to make medicines and the remaining part can be used for new planting

(Caldwell *et al*., 2016; Chand, Malik, & Nautiyal, 2016; Kumar *et al.,* 2013; Punetha *et al*., 2006).

Disease and pest Management

Powdery mildew occurs on the plants during the early growth period mainly in lower altitudes. There is a dearth of literature on control of powdery mildew by organic formulations in *P. kurroa* although milk or whey either alone or in combination with canola oil reduced severe powdery mildew condition in grapevines . Neem oil extract (azadirachtin) and jojoba oil also reduced powdery mildew in dogwood (*Cornus florida*) and grapevines. The use of chemical fungicides reported in literature and spraying plants with Topsin-M (thiophinate methyl 0.1%) about15-20 days after initiation succeeded by another spray after 15 days is reported for this plant. To prevent the seed loss due to insects and aphids it is advised to spray the plants with Ekalux 0.5 % (quinaphose) during the period of flowering and seed formation (Chand, Malik,& Nautiyal, 2016).

Crop production and commercial viability

Cultivation of this crop is sparse compared to other crops; a very few numbers of farmers are growing it in small areas of the Himalayas. Yield depends on the altitude and the cultivation practices. It is observed that at lower altitude of about 2000 m, yield of dry rhizomes is approximately 400-500 kg/ha and at altitude of above 2500 m yield is approximately 600 kg/ha (Caldwell *et al*., 2016).

Post-harvest management

The harvested materials should be washed properly and then dried at room temperature (15-25 ºC) to retain the active constituents. Drying of roots and stolons in the direct sunlight and oven should be avoided since this reduces the percentage of active constituents. The dried materials should be stored in a closed bag in a moisture free condition (Chand, Malik, & Nautiyal, 2016; Kumar *et al.,* 2013; Punetha *et al.,* 2006).

1.6. Adulteration

In spite of herbal medicines over many centuries as important mean for medication for treatment of illness or as dietary supplements and health products, little attention has been given towards the standardization of medicinal plants and other natural products. From the time of collection of plant or plant parts to its storage and upto the production of

medicines/formulations, chances of deterioration in quality are quite frequent; resulting in decline of efficiency of drugs. The herbal drugs can be used as a therapeutic agent only if they are genuine and their standard and quality are up to the mark. A disease can not be managed comprehensively until the delivery of genuine sample of drug is ensured. To protect the public health, a number of quality standards have been developed to safe guard against hazardous contamination and other abnormalities of these medicinal materials. These include determination of heavy metals, pesticide residues, mycotoxins, foreign matter, ash, extractives, water content etc. in the products. Of various quality related tests, the level of ash content and extractive values remain one of the most facile means to ascertain the quality of medicinal plant materials. Certain pharmacopoeias and some publications from World Health Organization (WHO) related to quality control aspects of medicinal plants have strongly emphasized these parameters as a requirement for quality standards. Herbal drugs are adulterated by a variety of ways:-

1) replacement by exhausted drugs
2) substitution by superficially similar,but inferior parts
3) substitution by artificially manufactured substitutes,
4) substitution by substandard commercial varieties
5) substitution by organic matter (non official parts) obtained from the same plant.

To maintain the safety, efficacy and quality of such products, a proper process for the authentication of crude drugs is needed. The general methods of authentication to check for adulteration of herbal drugs includes morphological/anatomical characterization, organoleptic markers (odor, color, texture) and chemical testing (Jahan *et al.,* 2008; World Health Organization, 1999; Thani *et al.,* 2018; Khatoon *et al.,* 1993; Dubey *et al.,* 2004) .

1.7. Phytoconstituents of *Picrorhizakurroa*

Phytochemical studies reveals that drug contain mixture of phytoconstituents. The phytochemical nature of *P. kurrooa*has been extensively studied andvarious examinations have led to the identification of 132 active ingredients from different parts of the plant such as roots, stem, leaf and seeds.The major chemical constituents are D-mannitol, Kutkiol, Kutki sterol, apocyanin, phenol glucosides, and rosim, and picein iridoid glycosides; kutkin, picrosideI, II & III; Kutkoside, minecoside, picrorhizin, arvenin picrosides present in kutkin are picrosideI and II which are ideally iridoidglycosides. Other

active constituents are drosin, apocynin, andnine cucurbitacin glycosides whereas catechol - apocynin has been found active as an anti-inflammatory agent and cucurbitacins for antitumor and cytotoxic effects. Apocynin (catechol) is anti-inflammatory in nature and prevents oxidative burst of neutrophil. As per literature there are several bioassays on the constituents isolated from the rhizomes focused on hepatoprotective, immune-modulating and antioxidant activities. (Rathee et al., 2012; Sharma et al., 2012; Thapliyal et al., 2012; Salma et al., 2017; Simons et al., 1990; Stuppner & Wagner, 1989; Weinges, Kloss & Henkels, 1972; Kumar *et al.,* 2013; Singh, 2004; Usman *et al.,* 2012).

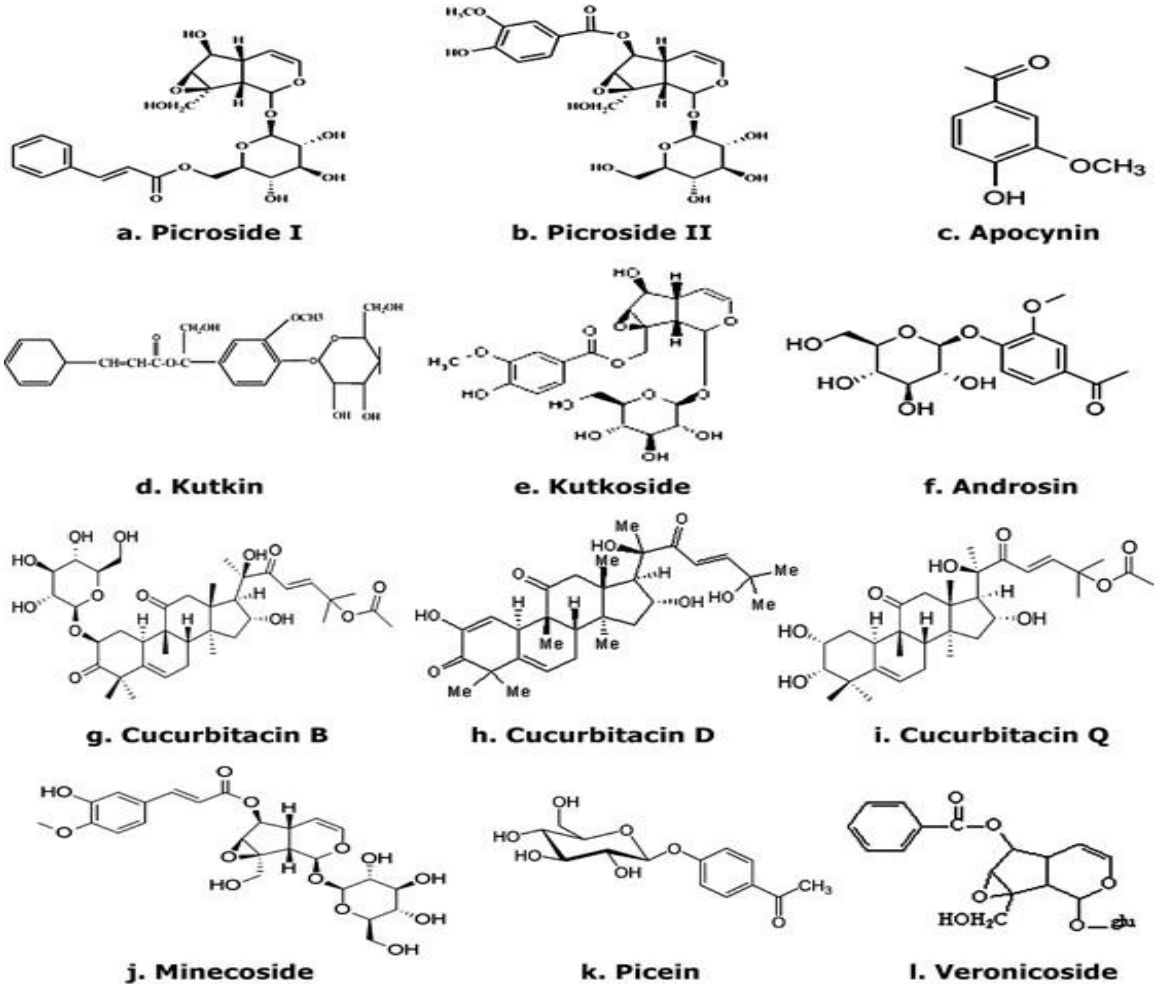

Fig.3 - Major Chemical constituents of *Picrorhiza kurroa*

1.8. Extraction Method

Extraction forms the first basic step in medicinal plant research because the preparation of crude extracts from plants is the starting point for the isolation and purification of chemical constituents present in plant. Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. The purpose of standardized extraction procedures for crude drugs is to attain the therapeutically desired portion and to eliminate unwanted material. The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultra sound extraction (Sonication), super critical fluid extraction and phytonic extraction (Handa *et al.,* 2008). In addition to this, the basic parameters influencing the quality of an extract are plant parts used as starting material, solvent used for extraction and extraction procedure (Ncube *et al.,* 2008). The selection of method to isolate active components with best yield and higher purity from natural sources is mainly dependent on the nature of compounds and raw material which is going to be processed (Kothari *et al.,* 2009). A typical extraction process includes, collection and authentication of plant material and drying, size reduction, extraction, filtration, concentration and drying and reconstitution (Thani *et al.,* 2018).

1.9. Storage conditions & Its Effect on Activity of Active Chemical Constituents

Medicinal properties of plants are manifested due to its bioactive compounds present in them. The stability of bioactive compound largely depends upon their chemical structure. Some of these compounds undergo degradation in the presence of heat, moisture, light, solvents and enzymes. The concentrations of these compounds in plants are greatly influenced by genotype, plant growth and development stages, and environmental factors. Immediately after harvesting the medicinal plants, it becomes important to minimize the loss of active constituents during drying conditions and storage of dried material till it is finally converted into formulation to be used by the consumers. Not much work pertaining to effect of different storage conditions and storage durations on stability of bioactive constituents of plant

Picrorhiza kurroa has been done. Furthermore, on their comparative study they have claimed that the loss in picroside-I and picroside-II with storage was maximum when "Kutki" samples were stored under humid condition (85% humidity, 25 °C) and minimum loss was observed when samples were stored at low temperature (4-6 °C). So, they suggested that to minimize the loss of picroside-I and picroside-II content in the drug Kutki during storage, Kutki must be stored at low temperature (Thani *et al.,* 2016).

2. Plant- *Inula racemosa* (*Ir*)

From ancient times various diseases have treated by using medicinal plants and phylogenic products throughout the world. Crude extracts are now considered a valuable source for natural products used in the development of medicines against various diseases for the development of pharmaceutical preparations and for novel biomedical research. The developed countries still depend on herbal traditional medicines. Even in developing as well as developed countries, herbal drugs play an important role in curing various human ailments. *Inula racemosa* (***Ir)*** mostly distributed in Asia in hilly areas. *Inula racemosa* is a powerful herb for ayurveda medical science and as well as pharma industry also. *Inula racemosa* Hook. F (Asteraceae) is a well-known medicinal plant in Ayurveda as Pushkarmula . First it mentioned as a variety of kustha in holy granth AGNI PURAN. Acharya Charak introduced it as hikka, swas, parswa shula hara (Bhramanand, 2015; Lucas, 2013). These plants have been used in treating different diseases like gastric irritability, febrifuge, nervous depression, anthelmintic, liver complients, jaundice, sores in eyes and fever etc. Now a days traditional medicine are used based on the availability of plant species, plant based products, economic affordability and effective against certain diseases when compared to modern medicine. (Ramalingam *et al.,* 2016; Charak Samhita, Acharya Vidyadhar shukla & Prof. Ravi Dutt Tripathi, 2018; Chunekar & Pandey, 2006). The plant is commercially is a very important medicinal plant as described in Ayurveda. In Ayurveda as Pushkarmula, which is widely used for *vatakapha jwara* (fever caused by vata pitta imbalance), *sotha* (swelling), *arachi* (anorexia), *swasa* (breathlessness) and *Parshwashoola* (chest pain on either side). It works as '*Rasayana'* (rejuvenator, immunomodulator) agent. The root powder is commonly used to cure *swasa roga* (asthma/ dyspnea), lipid disorder, angina and dyspnea, diabetes and to provide strength to heart by Ayurvedic practitioners in India. It possesses *Katu, Tikta rasa, Laghu guna, Katu Vipaka, Usna Virya*. The aqueous extract of the fresh or dry roots is given

orally in rats to evaluate glucose homeostasis and liver problems (Jaspreet & Pandey, 2016). Much of the investigations support the therapeutic efficacy of the plant as Cardioprotective, Anti-histamine, Antiallergic effect and Mast cell stabilizing, Antibacterial, Anti-diabetic, Adaptogenic, Antimutagenic, Antiapoptotic, Anti-inflammatory and Analgesic, Hepatoprotective activity and roots used as anthelmintic, antiseptic, anti-inflammatory and diuretic agents (Sharma *et al.*, 2006).

Fig.3: Leaves and Flowers of *Inula racemosa*

2.1. Botanical Description

Table 2.1: Taxonomy of *Inula racemosa*

Taxonomic Rank	Taxon
Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub class	Asteride
Order	Asterales
Family	Asteraceae
Genus	*Inula*

Species	*Racemosa*
Common Name	Pushkarmul

2.2.Vernacular Names

Inula racemosa hook f. is herb (plant) is commonly known as Puskaramula in Sanskrit and Pokharmula Hindi.). The regional names of *Inula racemosa* India are shown below:

Table 2.2: Vernacular Names of *Inula racemosa*

English	Indian elecampane, sunspear
Hindi	Pokharmul
Sanskrit	Puskara mula, kasmira, padma, sugandhikam
Telugu	Pushkara mulamu
Gujrati	Pokharmul
Kannada	Puskarmul
Cashmini	Poshkar
Malayalam	Puskkarmulam
Marathi	Pushkarmul
Tamil	Puskarmulam
Assamese	Puskar,Puharkarmul
Punjabi	Pokhar Mool

2.3. Synonyms

Kasmira, Padmapatra, Pouskara, Kustha, Bheda , Ciram , Jiham, Tirtha, Dhira, Padma, Puskarahava, Brahmatirtha, Mulam, Vatahva, Phala Patraka, Viram, Swasari, Sugandhikam, Mula, Pushkarjata (Chunekar & Pandey, 2006).

2.4. Ayurvdic Properties

Ras – Tikta, Katu

Guna – Laghu, Tikshna

Virya – Ushna

Vipaka – Katu

Karma – Kapha Vatahara, Hrdya, Dipana. (Manish & Omprakash, 2018)

2.5. Habitat & Geographical Distribution

Inula racemosa is distributed at temperate and alpine western Himalayas at an altitude of 5000 to 14000 ft. from Kashmir to Kumaon, Afghanistan to Nepal. It also found wild among strong alpine scrub vegetation in cold Laddakh (Leh) region of Kashmir (Kirtikar & Basu, 1991). It is a critically endangered species due to the fragile nature of its habitat and its exploitation due to commercial medicinal properties. The species is facing the onslaught of indiscriminate over exploitation, habitat destruction and competition (Shashi & Rajendra Kumar, 2012).

2.6. Botanical Description

Plant is a tall, stout herb stem is 0.3 to 1.5 mt, high, rough, groved and all vegetative parts are scabrid to mentose.

Fig.4: Flowers of *Inula racemosa*

a) Leaves

Basal leaves are 20 - 45 x12.5 – 20 cm. Lower leaves are narrowed to a winged leaf stack. Upper leaves are lanceolate and stem clasping. The abaxial laminal face is densely to mentose. Radical leaves are broad 20-45 x12.5 -20 cm. broad and elliptic lan shaped with long stalked. The cauline leaves are smaller, oblong and semi aplexicaule. Stem leaves oblong, half stem clasping usually deeply lobed at the base.

b) Flowers

Flower heads are shady yellow in colour produced in mid to late summer. Flowers in heads /racemes, very large 3.8 to 5 cm in diameter Outer bracts broad, tips triangular, bent back, inner bracts linear, sharp pointed. Flowering occurs from January to July. These borns on apical spike like cluster.

c) Fruits

Fruits (achenes) occurs in October to November months & fruits are cylinder, hairless, achne 4 mm long. Pappus 8 mm long and reddish in colour .

d) Root

Root stock branched, fresh roots are irregularly fusiform. Roots have a dull brownish skin with yellowish colour inside the possess a sweet and somewhat camphoraceous odour and have a bitter taste.

2. LITERATURE REVIEW (PHARMACOLGICAL ACTIVITIES)

Picrorhiza kurroa is an important herb in the Indian medical system of Ayurveda. It has been found to posses 'hepatoprotective, antioxidant, anti-inflammatory ,immunomodulatory

properties along with antiallergic, antiasthamatic, anticancerous, cardiovascular, choleretic, hypoglycemic, hypolipidemic, antiviral, purgative, anti-phosphodiesterase, neuritogenic, molluscicidal, and leishmanicidal activities . Its' underground part has been used traditionally for Asthma, bronchitis, Malaria, chronic dysentery, viral hepatitis, upset stomach, Scorpion sting, as a bitter tonic (stimulating the appetite and improving digestion), and as a liver protectant. Besides, it has been used in the treatment of skin conditions, peptic ulcer and neuralgia, vitiligo and rheumatic arthritis . Recently its' root and rhizome extracts of have also shown to possess anti malarial activity.

2.1. Anticancer

Kumar R et al., conducted an *in- vivo* study on BALB/c mice models for the evaluation of anti-cancer activity of *Picrorhiza kurroa*. Sarcoma was induced by 20-methylcholanthrene (20-MC) in models and papilloma formation was initiated by 7,12- dimethylbenz [a] anthracene (DMBA)in the models. The oral administration of picroliv (100 and 200 mg/kg, p. o) showed inhibitory actions against 20- MC and DMBA by decreasing sarcoma and papilloma. This study suggested that picroliv is a potent anti-cancer agent (Kumar R & Kuttan, 2001; Khandekar *et al*., 2019).

2.2. Anti-asthmatic

As per the report of Sehgal et al., *in- vivo and in-vitro* study, *Picrorhiza kurroa* isassociated with anti-asthmatic properties. They used guinea pig models for the study. For *in-vivo* study histamine was used to induce broncho-constriction in the models.It was revealed from the study that ethanolicextract of the roots showed protective actions. Whereas for *in-vitro* study same extract was studied on isolated guinea pig ileum. Protective actions of ethanolic extract were observed which demonstrated that *Picrorhiza kurroa* is associated with anti-asthmatic properties (Dorsch *et al*., 1991; Mahajani & Kulkarni,1977).

2.3. Anti-microbial

Antimicrobial potential of *Picrorhiza kurroa* was evaluated by Usman et al. They conducted an *in-vitro* study on bacterial starins i.e *Bacillus subtilis and Staphylococcus aureus* gram positive bacteria and *Pseudomonas aeruginosa and Escherichia coli* gram negative bacteria and *Aspergillusniger, Candida albicans and Malasseiza furfur* fungal strains. It was observed that ethanolic extract of this plant showed efficient action against all the used strains of microbes which suggests its use an anti-microbial. Antimicrobial activity of *Picrorhiza*

kurroa was also investigated by Sharma et al.It was observed that methanol extract showed more potent actions against bacterial strain (*E.coli ,B.subtilis ,S.aureus*)than antibacterial drug ciprofloxacin and aqueous extract was found to be more effective against fungal strain (*A. niger, C. albicans*) than Fluoconazole which is a standard antifungal drug. (Kumar & Singh, 2013; Verma *et al.*,2009; Zhang *et al.* 2012)

2.4. Anti-oxidant

Kalaivani et al., conducted an *in- vitro* study to evaluate the anti-oxidant and free radical scavenging potential of *Picrorhiza kurroa*. It was observed thatethanolic extract of this plant showed significant anti-oxidant properties due to thepresence of flavonoid and phenolic compounds (Khandekar *et al*., 2019)

2.5.Anti-mutagenic

As per the reported study conducted by Zaberi et al., hydroalcoholic extract of *Picrorhiza kurroa* is associated with anti-mutagenic actions. It was found that hydroalcoholic extract exhibited inhibitoryactions against Salmonella typhimurium MTCC 1251 and MTCC 1252 strains bydirect acting mutagen of sodium azide (Rathee et al., 2013; Soni & Grover 2019).

2.6. Immunomodulatory

As per the Hussain et al., *in-vivo study* report *Picrorhiza kurroa* is associated with immunomodulatory activities. The study was carried out on immunosuppressedmice models. Cyclophasphamide was induced in the models for immunosuppression. The study concluded that the alcoholic plant extract is significantly works on enhancement onimmunostimulant activities (Gupta *et al.*, 2006).

2.7. Anti-inflammatory

An *in-vivo* study carried out byKumar et al., on rat models to evaluate the Anti-inflammatory activities of *Picrorhiza kurroa,* suggested that this plant is a potent source of anti-inflammatory drug. In the study models were administered with carrageenan for inducing paw edema andimplanted with cotton pellet for inducing granuloma formation. Rhizome extract of *Picrorhiza kurroa* in a dose dependentmanner effectively worked on the inhibition of carrageenan-induced paw edema andcotton pellet-induced granuloma formation. It was also observed that there was reduction in levels of inflammatory cytokines (TNF-α, IL-1β, IL-6) which is accompanied with increase in anti- inflammatory cytokine (IL-10) level in the serum and peritoneal macrophages (Kumar et al., 2016).

2.8. Hepatoprotective

Hepatoprotective activity of*Picrorhiza kurroa* was investigated by Shetty et al., on male Wistar rat models.Models were fed with 30% high fat diet for 2 weeks for inducing non-alcoholic fatty liver disease (NAFLD). The administration of hydroalcoholic extract for 4 weeks at the dosage of 200mg/kg and 400 mg/kg b.i.d., p.o. showed potent hepatoprotective actionsby restoring all the changes in the liver induced in the liver (Shetty *et al.*, 2010).

2.9. Anti-diabetic

Husain et al., conducted an *in-vivo* study on rat models to investigate the anti- diabetic potential of *Picrorhiza kurroa.* The models were administered with streptozotocin nicotinamide to induce diabetes mellitus. The oral administration ofstandardized aqueous extract effectively helped in restoring all the changes induced by streptozotocin nicotinamide which suggests *Picrorhiza kurroa* use as an anti- diabetic agent (Husain et al., 2009).

2.10. Analgesic activity

Rupali et al observed Analgesic activity of the plant was assessed by the treatment of alcoholic root extract. The analgesic activity was assessed by employing the Hot plate and Acetic acid induced-writhing technique in albino mice of either sex. The 500mg/ kg extract dose of *P.Kurroa* had shown comparable effect in comparison to the standard drug pentazocin when kept for ½ hr *(Rupali et al., 2013)*

2.11. Cardioprotective effect

Nandave et al in 2013 studied normal rat pre-treated with *P. kurroa* (200 mg/kg) alone did not show noteworthy change; however, application of isoproterenol leads to hemodynamic and left ventricular dysfunction, lipid peroxidation and oxidative stress. Such type of cardiac dysfunction was considerably prohibited by the plant's root extract. Pre-treatment with root extract significantly checked the isoproterenol-induced oxidative stress by renovating various enzymes like myocardial superoxide dismutase, catalase and glutathione inlipid peroxidation, which prevent the outflow of myocyte creatine kinase-MB and lactate dehydrogenase enzymes (Nandave *et al*., 2013).

2.12. Antibacterial activity

Isoalantolactone, a major constituent of *I. racemosa* exhibit significant fungistatic nature of toxicity against *Gaeumannomyces graminis* var.*tritici*, *Rhizoctonia cerealis* and

Phytophthora capsici. It also shows weak antibacterial activities against *Staphylococcus aureus, Escherichia coli, Sarcina lentus, Pseudomonas fluorecense,* and *Bacillus subtilis.* Essential oil from I. racemosa also possesses antimicrobial potential (Kumar & Verma, 2020)

2.13. Anti-histamine, Anti allergic Effect and Mast cell stabilizing activity

Inula racemose was investigated against different type of stimulus for asthma such as immunological, physiological and biochemical conditions. Petroleum ether extract exhibit superior activity then other ethanol and water extract. Petroleum ether extract found to be effective against histamine induced contraction, milk induced leucocytosis, milk-induced eosinophilia and clonidine- induced mast cell. Srivastava has reported that alcoholic extract of root of *I. racemosa*, shows significant antiallergic effect in egg albumin induced passive cutaneous anaphylaxis (PCA) and mast cell degranulation in albino rats.

2.14. Cardioprotective activity

I.racemosa alcohol extract significantly reverse the lipid derangement and reduced the atherogenic index. It also shows antioxidant potential in heart. It prevents Coronary artery changes, Aortic lesion area and % bodyweight increase.In a study it was reported that *I. racemosa* root adrenergic beta-blocking activity as it prevent ST-segment depression, T-wave inversion in the post-exercise electrocardiogram and exhibit negative inotropic as well as negative chronotropic effects on frog heart. It was also reported that it prevent myocardium from isoproterenol-induced infarction and leakage of myocytes specific marker enzymes creatine phosphokinase-MB and lactate dehydrogenase from the heart. Moreover it improved cardiac function by increasing the heart rate, mean arterial pressure, contractility and relaxation along with decreasing left ventricular end diastolic pressure.

2.15. Anti-diabetic activity

The petroleum ether extract of roots lowered plasma insulin and glucose levels within 75 min of oral administration to albino rats and it significantly counteracted adrenaline-induced

hyperglycaemia in rats. Alcoholic extract of the roots of *I. racemosa* exhibit hypoglycemic effect along with increase in liver glycogen content. However it does not induce insulin secretion from pancrease. Aqueous and methanol extract of the root of I. racemosa produced significant reduction in blood sugar level in glucose included hyperglycemia and alloxan-induced hyperglycemia respectively (Husain *et al.,* 2009)

2.16. Adaptogenic activity

The animals treated with root extract *I. racemosa* (100mg/kg) and (200mg/kg) showed positive adaptogenic activity. It significant reduction in the immobility period in forced swimming test as well as elevates anti-oxidant markers in addition to adrenaline and serotonin levels.

2.17. Antimutagenic and Antiapoptotic Effects

Aqueous root extract (ARE) of *I. racemosa* was experimentally investigated for its chemopreventive potencial. It was found effective against 4-nitroquinoline-1-oxide (4-NQO) induced DNA damage and apoptosis in mice bone marrow cells.

2.18. Anti-inflammatory and Analgesic activity

Anti-inflammatory (carrageenan induced paw edema) and analgesic (hot plate) activity was performed with Ethanolic extracts of *I. racemosa*. The results were impressive as compared to standard drug Aspirin as well as indomethacin.

2.19. Hepatoprotective activity

The aqueous, methanolic and total aqueous extract showed better hepatoprotective activity than the petroleum ether extract at 100 mg/kg in rats and they were non toxic upto 10 g/kg. The LD50 of pet ether extract is reportedly 1.5g/kg bw. Hydro-alcohol extract of root of *I. racemosa* shows free radical scavenging activity in DPPH activity, reducing power and hydrogen peroxide scavenging activity. In vitro hepatoprotective activity of *I. racemosa* roots against CCL4 induced toxicity on chang cell Line. Higher percentage viability was observed with ethanolic extract treatment. It was also reported that the drug shows liver-protective activity against Hepatic ischemia–reperfusion (I/R). It has ability to restore normal hepatic architecture (Shetty *et a*l., 2010).

3. RESEARCH ENVISAGED

3.1. PLAN OF WORK

S. No.	WORK	MONTH
1.	Submission of proposed title	Dec.2021
2.	Literature survey	15th May, 2022
3.	Synopsis submission	30th May, 2022
4.	Experimental	June-2022 onwards

4. OBJECTIVES

The aim of present investigation is to:

4.1. Extraction of Hepatoprotective Plant Leaves- *Picrorhiza kurroa and Inula racemosa*

4.2. In Vitro Hepatoprotective Activity

4.3. Herbal Formulation

5. PROPOSED METHODOLOGY

Aim is to investigate the total active ingredients from the leaf part of Picrorhiza kurroa by using suitable solvent by extraction method. In Vitro Hepatoprotective Activity of the drug will be done on cell lines and will formulate herbal drug. Beside this isolation of noble drug will also to be done.

5.1 Materials and Methods

Chemical- All chemicals will be obtained from SD Fine Chemicals, Mumbai.

Plant Material

Mature leaves of P'kurroa will be collected from the Himalayan region near the Rohtang pass around the village Kothi in Himachal Pradesh in summer season in the month of May to September.

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CERTIFICATE
(SYNOPSIS APPROVAL CERTIFICATE)

This is to certify that the Synopsis/Revised Synopsis for Ph.D. Thesis titled "**In Vitro Hepatoprotective Activity from Leaf Part of *Picrorhiza kurroa* and *Inula racemosa* and Its Herbal Formulation**", submitted by **Ms. Rekha Rana** to the Carer Point University, Hamirpur (H.P.), India in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in the **Department of Pharmaceutical Sciences, School of Pharmaceutical and Health Sciences** has been approved by the Research Advisory Committee (RAC) after an Oral Examination of the student on dated ...30 May 2022.

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